AMENDMENTS TO THE SPECIFICATION

- Please insert the following two (2) paragraphs on page 5 at the end of section entitled "Brief Description of the Drawings"

Fig. 2a-d depicts Flowchart 1, which shows the Process Flow Diagram for recovery and purification of B-2036 as exemplified in Examples 1 & 2.

Fig. 3a-c depicts Flowchart 2, which shows the Process Flow Diagram for recovery and purification of B-2036 as exemplified in Examples 3 & 5.

- Please replace Example 1 on pages 76 & 77 with the following amended Example 1.

Example 1

Decrease of Trisulfide Isoform Impurity of
Growth Hormone Antagonist (GHA) with Mercapto Compound(s)

Reduction of B-2036-trisulfide level was accomplished using Retentate 1 (see flowchart 1 ~~below~~ **in Figure 2a-d**) in the B-2036 purification process. Fermentation of the recombinant *E. coli* expressing B-2036 was carried out as described by Cunningham et al. in US Pat. No. 5,849,535. The initial extraction and purification steps (all steps conducted prior to obtaining Retentate 1 in flowchart 1 ~~below~~ **in Figure 2a-d**, e.g., all resuspension and homogenization, two-phase extraction, reversed phase chromatography, and anion exchange chromatography) were carried out as described in the accompanying flowchart 1 **Figure 2a-d**). Following the first ultrafiltration/diafiltration step, the product was present in 25 mM HEPES, pH 7.0 buffer (~150 L) at a protein concentration of 3.7 g/L. The temperature of the product solution was cooled to 2 to 8°C and it was treated with a $1/10^{th}$ volume addition (~15 L) of freshly prepared 200 mM Tris, 20 mM cysteine, pH 8.0 buffer that had been cooled to 2 to 8°C. The B-2036/cysteine solution was maintained at 2 to 8°C and mixed for 174 minutes. The mixture was then concentrated to 131 L using Millipore

Biomax 5 ultrafiltration membranes and diafiltered against a 6X volume of 25 mM HEPES, pH 7.0. The resulting product was then carried through the remainder of the B-2036 purification process as described in flowchart 1 ~~below~~ **in Figure 2a-d**.

Before the cysteine incubation, the B-2036/trisulfide level was measured to be 3.7 area percent. Immediately after the cysteine incubation, its level dropped to 2.0 area percent (decreased by about 46%).

- Please delete pages 78, 79, 80, & 81 comprising Flowchart 1 objected to by the Examiner.

- Please replace Examples 2 & 3 on pages 82 & 83 with the following amended Examples 2 & 3.

Example 2

Decrease of Trisulfide Isoform Impurity of
Growth Hormone Antagonist with Chelating Agent(s)

Referring to flowchart 1 **(Figure 2a-d)**, 165 Kg of frozen GHA cell paste cell paste were added to ~1013 liters of 150 mM Tris, 5 mM EDTA, pH 7.2. When the cell paste had finished thawing (determined by stable A_{550} readings) the mixture was passed through a Niro homogenizer at 950 +/- 50 Bar at a nominal flowrate of 250 l/hour. The homogenate was collected in a tank where the temperature was maintained between 24 and 33°C. Ammonium sulfate and PEG-4600 were added to the lysate mixture to form a concentration of 10% (w/w) of both compounds. The resulting two-phase mixture was mixed for 60-120 minutes and then these phases were resolved by passing the solution through a liquid/liquid, solids discharging centrifuge. The top phase contained the desired product and was collected and filtered through a filter train consisting of a "delipidating" filter, "depth" filter , and a 0.2 μm filter. The filtrate was collected in three separate totes and samples of each were analyzed for B-2036-trisulfide. The levels ranged from 3.1 to

3.8 area percent. The resulting material was then processed to bulk intermediate using the procedure outlined in the flowchart 1 **(Figure 2a-d)** ~~of Example #1~~.

By comparison, samples from lots processed by the above-noted procedure (but without the EDTA component (chelating agent) in the lysis buffer and without cysteine treatment of Retentate 1) yielded a trisulfide level (n=10) ranging from 3.2 to 6.4 area percent with a mean of 5.1 at the end of the entire process of flowchart 1 ~~above~~ **(Figure 2a-d)**. When EDTA was included in the lysis buffer, and the cysteine incubation step of retentate 1 was conducted, the trisulfide level at the end of the entire process of flowchart 1 was 1.5% area percent.

Example 3

Decrease of Trisulfide Isoform Impurity of
Growth Hormone Antagonist with Metal Salt(s)

Referring to flowchart 2 ~~below~~ **(Figure 3a-c)**, 165 Kg of frozen cell paste cell paste were added to ~1013 liters of 100 mM sodium phosphate, pH 6.0. When the cell paste had finished thawing (determined by stable A_{550} readings) the mixture was passed through a Niro homogenizer at 950 +/- 50 bar at a nominal flowrate of 250 l/hour. The homogenate was collected in a tank where the temperature was maintained between 24 and 33°C. Ammonium sulfate and PEG-4600 were added to the lysate mixture to form a concentration of 10% (w/w) of both compounds. The resulting two-phase mixture was mixed for 60-120 minutes and then these phases were resolved by passing the solution through a liquid/liquid, solids discharging centrifuge. The top phase contained the desired product and was collected and filtered through a filter train consisting of a "delipidating" filter, a "depth" filter, and a 0.2 μm filter. The material was carried through the remainder of the B-2036 process as described in flowchart 2 ~~below~~ **(Figure 3a-c)**. The level of B-2036-trisulfide present at the end of processing was 1.4 area percent. This compared with B-2036-trisulfide levels of 3.2 to 6.4 area percent (mean = 5.1 area percent) for B-2036 lots (n=10) processed with the normal lysis buffer (150 mM Tris, pH 7.2).

- Please delete pages 84, 85 & 86 comprising Flowchart 2 objected to by the Examiner.

- Please replace Examples 4 & 5 on pages 87 & 88 with the following amended Examples 4 & 5.

Example 4

Decrease of Des-Phe Isoform Impurity of
Growth Hormone Antagonist with Chelating Agent(s)

Referring to flowchart 1 **(Figure 2a-d)**, 165 Kg of frozen cell paste cell paste were added to ~1013 liters of 150 mM Tris, 5 mM EDTA, pH 7.2. When the cell paste had finished thawing (determined by stable A_{550} readings) the mixture was passed through a Niro homogenizer at 950 +/- 50 Bar at a nominal flowrate of 250 l/hour. The homogenate was collected in a tank where the temperature was maintained between 24 and 33°C. Ammonium sulfate and PEG-4600 were added to the lysate mixture to form a concentration of 10% (w/w) of both compounds. The resulting two-phase mixture was mixed for 60-120 minutes and then these phases were resolved by passing the solution through a liquid/liquid, solids discharging centrifuge. The top phase contained the desired product and was collected and filtered through a filter train consisting of a "delipidating" filter, a "depth" filter, and a 0.2 μm filter. The filtrate (obtained after top phase filtration; see flowchart 1) was collected in three separate totes and samples of each were analyzed for B-2036/des-phe. The levels ranged from 3.2 to 6.3 area percent. For comparison, further processing according to flowchart 1 **(Figure 2a-d)** further reduced the B-2036 des-phe level to 0.3 area percent of Retentate 3. This further reduction of B-2036 des-phe level was achieved by the product collection parameters of the second anion exchange chromatography step. When the lysis buffer included EDTA and the collection procedures of the ion exchange chromatography step were conducted, B-2036/des-phe levels of 0.3 are a percent were obtained as compared to the final process levels of 4.6 to 16.2 area percent (mean = 10.2 area percent) for B-2036 lots (n=10) processed without EDTA (e.g., 150 mM Tris, pH 7.2) and without the above-noted collection procedures.

Example 5

Decrease of Des-Phe Isoform Impurity of
Growth Hormone Antagonist with Metal Salt(s)

Referring to flowchart 2 **(Figure 3a-c)**, 165 Kg of frozen cell paste cell paste were added to ~1013 liters of 100 mM sodium phosphate, pH 6.0. When the cell paste had finished thawing (determined by stable A_{550} readings) the mixture was passed through a Niro homogenizer at 950 +/- 50 bar at a nominal flowrate of 250 l/hour. The homogenate was collected in a tank where the temperature was maintained between 24 and 33°C. Ammonium sulfate and PEG-4600 were added to the lysate mixture to form a concentration of 10% (w/w) of both compounds. The resulting two-phase mixture was mixed for 60-120 minutes and then these phases were resolved by passing the solution through a liquid/liquid, solids discharging centrifuge. The top phase contained the desired product and was collected and filtered through a filter train consisting of a "delipidating" filter, a "depth" filter , and a 0.2 μm filter. The filtrate was collected in three separate totes and samples of each were analyzed for B-2036/des-phe. The levels ranged from 6.0 to 9.4 area percent. For comparison, further processing according to flowchart 2 further reduced the B-2036/des-phe level to 0.8 area percent of Retentate 2. This further reduction of B-2036/des-phe level was achieved by the product collection parameters following the second anion exchange chromatography step. When the lysis buffer included sodium phosphate and the collection procedures of the second ion-exchange chromatography step were conducted, the B-2036/des-phe level of 0.8 area percent was obtained as compared to the final process levels of 4.6 to 16.2 area percent (mean = 10.2 area percent) for B-2036 lots (n=10) processed without sodium phosphate (e.g., 150 mM Tris, pH 7.2) and without the above-noted collection procedures.